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IP Journal of Diagnostic Pathology and Oncology

Journal homepage: https://www.jdpo.org/

Original Research Article

# Evaluation of expression of immunohistochemical markers high molecular weight cytokeratin (HMWCK) and alpha-methylacyl coa racemose (AMACR) in prostatic needle biopsies and transurethral resection of prostate (TURP) specimen – A one year observational study

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ARTICLE INFO

*Article history:*
Received 30-10-2023
Accepted 15-01-2024
Available online 31-01-2024

*Keywords:*
Prostatic Adenocarcinoma
PIN (prostatic intraepithelial neoplasia)
IHC (immunohistochemistry)
AMACR
HMWCK

ABSTRACT

**Context:** In view of increasing incidence of Prostate cancer with age, its early detection and management is of utmost importance. Digital rectal examination, clinical picture and USG findings are non-specific. In prostatic lesions having a suspicious morphology, IHC staining (HMWCK and AMACR) is done to distinguish benign from malignant lesions. Absence of myoepithelial layer (HMWCK negative) along with cytoplasmic granular staining in glands (AMACR positive) is consistent with malignant diagnosis.
**Aims:** To evaluate the utility of IHC markers HMWCK and AMACR in resolving morphologically suspicious foci on Prostatic needle core biopsies and TURP specimens.
**Settings and design:** Observational Study
**Materials and Methods:** A total of 30 cases of prostatic lesions were studied. The specimens were fixed in 10% formalin and routinely processed. Haematoxylin-Eosin (H&E) and IHC staining (HMWCK and AMACR) was done in all 30 cases.
**Statistical analysis used:** Data collected was analyzed using appropriate statistical test.
**Results:** A total of 30 cases including 19 cases prostatic needle core biopsies and 11 cases of TURP specimens were included in our study. Histopathological diagnosis included 1 case each of Adenosis, Atypical adenomatous hyperplasia and Transitional cell metaplasia; 9 cases of BPH with suspicious foci, 4 cases of LGPIN, 3 cases of HGPIN and 11 cases Prostatic adenocarcinoma. In 5 cases including 3 cases of BPH with suspicious foci and 1 case each of adenosis and AAH, the diagnosis was changed to Prostatic Adenocarcinoma after IHC analysis.
**Conclusion:** We conclude that IHC staining should be done in cases where routine H&E sections have an ambiguous morphology. HMWCK along with AMACR is a good marker combination to differentiate Benign from Malignant lesions.



## 1. Introduction

Prostate adenocarcinoma is a leading cause of morbidity and mortality worldwide affecting 1 out of 9 men over 65 years and being the second most common malignancy in men. The incidence of prostatic carcinoma is increasing with age. It rises from 20% in males in their 50s to 70% in men aged 70 to 80 years. [1]

Clinical presentation of benign prostatic hyperplasia (BPH) and carcinoma prostate are same - retention of urine, dysuria, frequency, urgency, backache, hematuria, etc. Due to their posterior position, a rectal examination can

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https://doi.org/10.18231/j.jdpo.2024.009

identify some early prostatic carcinomas, however the test has limited sensitivity and specificity.[2]

Ultrasonography has some characteristic findings of BPH and carcinoma prostate, but poor sensitivity and specificity limit its diagnostic utility. The prostate specific antigen (PSA) has proven to be helpful in the detection and treatment of prostate cancer, though PSA is not cancer specific but organ specific.[2]

In view of increasing trend of the occurrence of both neoplastic and non-neoplastic lesions of the prostate in the elderly, the current study aims at evaluating the histomorphological features of Transurethral Resection specimens of prostate (TURP) and prostatic needle core biopsies for a period of one year. Use of immunohistochemical markers – HMWCK and AMACR in this study helps in arriving at diagnosis and to differentiate between benign and malignant lesions of prostate. Absence of basal cell layer (HMWCK negative) along with cytoplasmic granular staining in glandular cells (AMACR positive) is consistent with malignant diagnosis.

## 2. Materials and Methods

This was a hospital based – observational (prospective) study in which 30 cases of morphologically suspicious TURP specimens and needle core prostatic biopsies were taken. The study was done for a period of one year i.e., from $1^{st}$ January 2020 to $31^{st}$ December 2020. Sample size was taken as 30 as per universal sampling method. Consent from the patients was taken, and the clinical history as well as results of relevant investigations were collected. The specimens were received at Department of Pathology in our hospital and were fixed in 10% formalin solution and were routinely processed. All 30 cases were stained with Haematoxylin-Eosin (H&E) for studying histopathological features and were further subjected to IHC staining using HMWCK and AMACR for confirmation of initial diagnosis.

HMWCK shows discontinuous, intact, circumferential staining of basal cells in benign and pre-malignant lesions but absent staining in malignant lesion.

Dark diffuse or granular, cytoplasmic, or luminal, but circumferential staining signifies AMACR positivity. The positivity is graded from 0 to 3+ as shown in Table 1.

The statistical analysis was done using SPSS 28 version software system. Results were expressed in numbers and percentages along with graphs and charts. The predictive values (positive and negative) were calculated after analysis of true negatives, true positives, false negatives, and false positives. Kappa statistics was done for studying the true agreement of using IHC markers for differentiating benign lesions from malignant ones.

## 3. Results

This was an one-year observational study of 30 male patients with prostatic disease having suspicious morphology on histopathological examination. The expression of IHC markers HMWCK and AMACR was studied to arrive to a definitive final diagnosis.

Out of the 30 cases in this study, the initial Histopathological diagnosis revealed cases of Adenosis, Atypical Adenomatous Hyperplasia (AAH), Transitional Cell Metaplasia (TCM), BPH with suspicious focus, PIN (LGPIN and HGPIN) and Prostatic Adenocarcinoma. (Table 2)

In our study, age ranged from 60-88 years and Mean Age ± SD was 74.53±7.41 years. Majority (16 cases, 53.3%) of cases were in the 70-79 years age group. Out of 30 cases, 19 cases (63.3%) were Prostatic needle core biopsy specimens and remaining 11 cases (36.7%) were TURP chips. PSA levels were correlated according to the histopathological diagnosis. Mean PSA level was highest in Prostatic adenocarcinoma while TCM has the lowest value followed by AAH. (Table 3)

In the 11 prostate adenocarcinoma cases, histopathological features like Gleason's score, tumor volume and perineural invasion (PNI) were studied. Majority (6 cases, 54.5%) had Gleason's Score of 3 + 3 = 6. Out of 11 Adenocarcinoma cases, 27.2% (3 cases) show approximate tumor volume of <10% while 36.4% (4 cases) of 11-50% and 36.4% (4 cases) >50% tumor volume. Six cases (55%) had a prominent PNI while in remaining 5 cases (45%) PNI was not seen.

HMWCK immunostaining was done in all cases to highlight the presence or absence of basal cell layer and along with it AMACR staining in glands was studied to differentiate benign from malignant lesions. (Figure 1)

In the present study, one case each of AAH and Adenosis (Figure 2) showed HMWCK negative in basal cells as well as AMACR positivity in the glands; hence the diagnosis was changed to Prostatic Adenocarcinoma (False Negatives). Out of 9 BPH with suspicious focus cases (Figure 3), 3 cases show AMACR overexpression along with loss of basal cell layer (HMWCK negative) at the suspicious focus; these were changed to Prostatic Adenocarcinoma (False Negatives). Four cases of LGPIN showed intact basal layer i.e., HMWCK positive while AMACR showed no immunostaining. In all the 3 cases of HGPIN, HMWCK was positive while AMACR was positive in 2 out of 3 cases (Figure 4). Hence, in all cases of LGPIN & HGPIN, initial histopathological diagnosis was retained. Ten out of 11 cases of adenocarcinoma were HMWCK negative and AMACR positive (True Positives) and 1 case showed both HMWCK and AMACR negativity (False Negative). (Table 4 ; Figures 5 and 6)

According to our study, after analysis of both the IHC markers (HMWCK and AMACR), the Positive and

Negative Predictive value of using these IHC markers for a correct diagnosis is 100% and 73.7% respectively. Kappa statistics for true agreement was calculated which showed a kappa value ($\kappa$) of 0.67, which signifies Substantial Agreement for using IHC markers (HMWCK and AMACR) to differentiate Benign from Malignant lesions.

**Table 1:** Interpretation of AMACR staining

| % of stained cells | Grade | Interpretation |
|---|---|---|
| 0 | 0 | Negative |
| 1-10 | 1+ | MILD |
| 11-50 | 2+ | Moderate |
| >51 | 3+ | Strong |

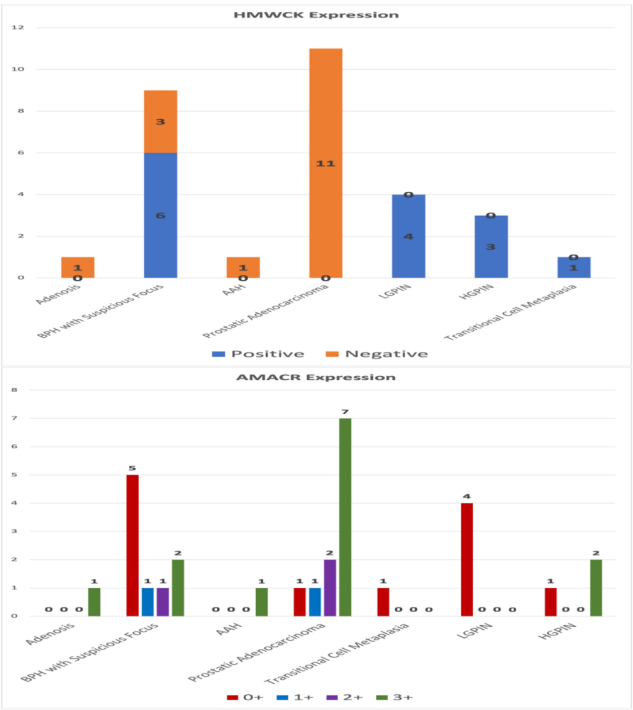


**Figure 1:** Expression of HMWCK and AMACR immunostaining in different cases

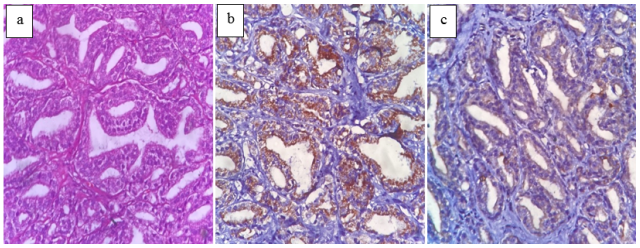


**Figure 2:** Adenosis **a**: H&E stain (400x) showing closely packed glands; **b**: AMACR 3+ (400x) showing cytoplasmic granular staining in the glands; **c**: HMWCK negative (400x) showing absent basal myoepithelial cell layer. The diagnosis of this case was changed to Prostatic Adenocarcinoma as AMACR was strongly positive with HMWCK negative.

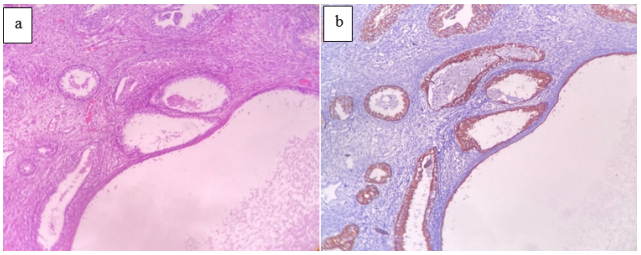


**Figure 3:** Benign Prostatic Hyperplasia (BPH); **a:** H&E stain (100x) showing cystically dilated glands; **b:** HMWCK stain (100x) positive - intact basal cell myoepithelial layer.

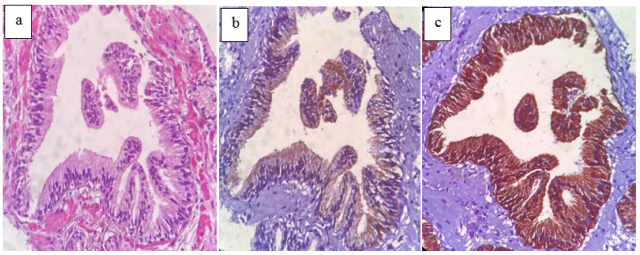


**Figure 4:** High Grade Prostatic Intraepithelial Neoplasia (HGPIN); **a:** H&E stain (400x) showing epithelial proliferations forming characteristic roman arches; **b**: AMACR 3+ (400x) showing cytoplasmic granular staining; **c**: HMWCK stain (400x) showing positive staining in both glandular epithelial cells and basal myoepithelial cells.

## 4. Discussion

The present study was carried out on 30 cases of Prostatic lesions on needle core biopsies and TURP specimens. These were examined for histomorphological lesions with suspicious morphology and further subjected to IHC marker staining using HMWCK and AMACR for confirmation of diagnosis.

In the present study, majority of cases were in the age group 70-79 years. The mean age for BPH with suspicious focus and PIN was 72.88 years and 74.04 years respectively, which is comparable to a study by Mwakoma HA[6] and, Pacelli A and Bostwick DG.[7] Prostatic adenocarcinoma is a disease of elderly. In our study mean age of adenocarcinoma was 75.62 years which is comparable to previously done studies.[6–10]

In studies done by Kumaresan et al[4] and Shah RB et al,[11] the incidence of BPH with suspicious focus ranged from 26 - 46%; in our study we found an incidence of 30%. Pre-malignant lesions like LGPIN and HGPIN in the present study had an incidence of 13.3% and 10% respectively which is similar to studies done by Rekhi et al[12] (11.2%) and Kumaresan et al8 (14.2%). The incidence of Prostate Adenocarcinoma was comparable to studies by Jasani et

**Table 2:** Distribution of cases according to histopathological diagnosis

| Histopathological Diagnosis | Number of Cases | Percentage (%) |
|---|---|---|
| AAH | 1 | 3.3 |
| Adenosis | 1 | 3.3 |
| BPH with Suspicious Focus | 9 | 30 |
| LGPIN | 4 | 13.3 |
| HGPIN | 3 | 10 |
| Transitional Cell Metaplasia | 1 | 3.3 |
| Prostatic Adenocarcinoma | 11 | 36.7 |
| TOTAL | 30 | 100 |

**Table 3:** PSA correlation according to histopathological diagnosis

| HP Diagnosis | Number of Cases | Minimum PSA (ng/mL) | Maximum PSA (ng/mL) | Mean PSA (ng/mL) |
|---|---|---|---|---|
| AAH | 1 | - | - | 6.75 |
| Adenosis | 1 | - | - | 8.63 |
| BPH with Suspicious Focus | 9 | 4.61 | 66 | 18.31 |
| LGPIN | 4 | 14.8 | 48.08 | 31.03 |
| HGPIN | 3 | 6.13 | 33.7 | 16.05 |
| TCM | 1 | - | - | 3.5 |
| Prostatic Adenocarcinoma | 11 | 6.91 | 180.35 | 39.4 |

**Table 4:** Comparison in change of diagnosis with IHC markers

| Initial Histopathological Diagnosis | Number of Cases | HMWCK (Basal Cells ) | AMACR (Glands) | Final Diagnosis |
|---|---|---|---|---|
| AAH | 1 | Negative | Positive | Prostatic Adenocarcinoma |
| Adenosis | 1 | Negative | Positive | Prostatic Adenocarcinoma |
| BPH with Suspicious Focus | 6 | Positive | Negative | BPH |
| | 3 | Negative | Positive | Prostatic Adenocarcinoma |
| LGPIN | 4 | Positive | Negative | LGPIN |
| HGPIN | 2 | Positive | Positive | HGPIN |
| | 1 | Positive | Negative | |
| Transitional Cell Metaplasia | 1 | Positive | Negative | TCM |
| Prostatic Adenocarcinoma | 10 | Negative | Positive | Prostatic Adenocarcinoma |
| | 1 | Negative | Negative | |

**Table 5:** Comparison of immunostaining HMWCK and AMACR in cases of BPH with suspicious focus

| Authors | % of HMWCK Positivity | % of AMACR Negativity |
|---|---|---|
| Garg et al[3] | 50 | 64.3 |
| Kumaresan et al[4] | 69.2 | 50 |
| Present study | 66.7 | 66.7 |

**Table 6:** Comparison of immunostaining HMWCK and AMACR in adenocarcinoma prostate cases

| Authors | % of HMWCK Negativity | % of AMACR Positivity |
|---|---|---|
| Molinie et al[5] | 86 | 97 |
| Kumaresan et al[4] | 84 | 92 |
| Garg et al[3] | 100 | 100 |
| Present study | 100 | 90.9 |

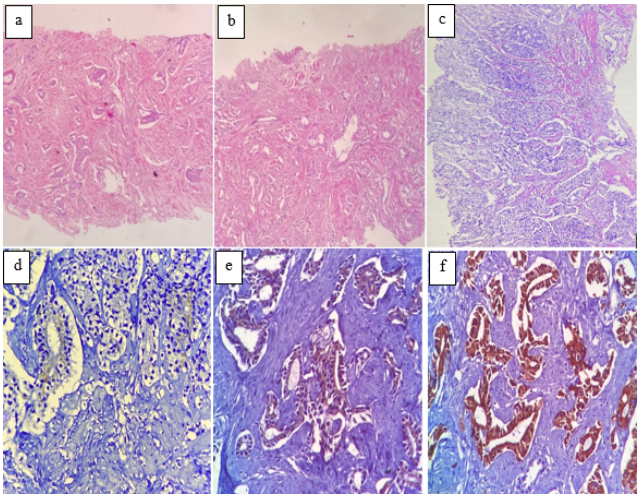


**Figure 5:** ProstaticAdenocarcinoma; **a-c:** H&Estain (100x) showing neoplastic glands with Gleason's score of (**a**) 3+3=6; b: 3+4=7, (**c**) 4+4=8; **d,e**: AMACR positivity (100x) in neoplastic glands graded as (**d**) 2+[11-50% cells stained]; e: 3+[>50% cells stained]; **f**: HMWCK staining (100x) showingpositive staining in the glandular epithelial cells and negative staining inbasal myoepithelial cells.

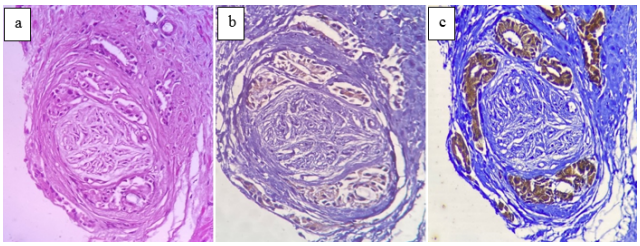


**Figure 6:** Perineural invasion (PNI) in case of Prostatic Adenocarcinoma; **a:** H&E stain (400x) showing a nerve entrapped and infiltrated by neoplastic glands; **b**: AMACR 3+ (400x) showing cytoplasmic granular staining in the neoplastic glands; **c**: HMWCK negative (400x) showing absent basal myoepithelial cell layer and positive staining in the glandular epithelial cells.

al,[13] Haroun et al,[14] Xie et al[9] and Shimada et al.[10]

A correlation between serum PSA levels and histopathological diagnosis was done in the present study which showed all the lesions have a raised (>4ng/mL) PSA levels with mean PSA value being higher in the Prostate Adenocarcinoma patients as compared to BPH and other pre-malignant entities. These results are consistent with the previously done studies as well.[15–17]

All 30 cases were stained using IHC markers HMWCK and AMACR. In the 9 cases of BPH with suspicious foci, 6 cases (66.7%) showed intact continuous basal cell layer as highlighted by HMWCK and lack of AMACR staining in the glands; while 3 cases (33.3%) had HMWCK negative staining in the suspicious focus along with AMACR expression in the glands which led to the change in diagnosis to prostatic adenocarcinoma. Comparison with previous studies is given in Table 5.

In the present study, all 7 cases (100%) of PIN (LGPIN and HGPIN) showed HMWCK positivity of basal myoepithelial cell layer. AMACR was negative in 4 cases of LGPIN and 1 case of HGPIN; while AMACR expression was observed in 2 cases (66.7%) of HGPIN. These results correlate well with previously done studies with similar findings as highlighted by Kunju et al[18], Jiang et al[19], Molinie et al[5], Kumaresan et al[4] and Kruslin et al.[17]

In the present study, out of 11 cases of carcinoma prostate, 10 cases (90.9%) showed positive AMACR overexpression (7 cases showing strong positivity, 2 moderate positivity while 1 case mild positivity) and, all cases were HMWCK negative. One case of prostatic adenocarcinoma showed both HMWCK and AMACR negativity. Similar results were observed in previously done studies by various authors. Comparison with other studies is given in Table 6.

## 5. Conclusion

Our study was an attempt to evaluate the expression of IHC markers HMWCK and AMACR in cases of prostatic lesions having suspicious or inconclusive morphological focus on routine H&E sections; resolving such dilemmas and arriving at a definitive diagnosis with the aid of IHC staining. In this study, we examined lesions of BPH with suspicious focus, PIN (LGPIN and HGPIN), cancer mimickers like AAH and Adenosis, as well as Prostatic Adenocarcinoma cases. All these cases were subjected to IHC analysis using HMWCK and AMACR. HMWCK was used to highlight the presence and intactness of basal myoepithelial cell layer giving a luminal and cytoplasmic staining; absence of HMWCK staining was suggestive of malignant diagnosis. AMACR positivity was noted in glands as circumferential cytoplasmic granular staining and was graded (0 to 3+) based on the percentage of cells stained.

Thus, we conclude that IHC staining should be done in such cases where routine H&E sections have an ambiguous morphology. HMWCK and along with it, AMACR is a good IHC pair to differentiate Benign from Malignant lesions especially when it comes to distinguishing HGPIN from adenocarcinoma. IHC is an aid to resolve such dilemmas and come to a confirmatory diagnosis so that early detection of cancer can be done and the patient is benefitted by appropriate management from the treating clinicians.

## 6. Ethical Clearance

The study protocol was approved by the institutional ethical committee JN medical college Belagavi, Karnataka. All participants signed an informed consent form prior to taking part in the study.

## 7. Source of Funding

None.

## 8. Conflict of interest

None.

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**Cite this article:** Kapoor R, Bannur HB. Evaluation of expression of immunohistochemical markers high molecular weight cytokeratin (HMWCK) and alpha-methylacyl coa racemose (AMACR) in prostatic needle biopsies and transurethral resection of prostate (TURP) specimen – A one year observational study. *IP J Diagn Pathol Oncol* 2024;9(1):49-54.